Performance of the Abbott RealTime HIV-1 assay versus the Roche Amplicor HIV-1 Monitor™ Test, v1.5, UltraSensitive assay for samples with low plasma HIV-1 RNA copy numbers

Christoph Stephan1*, Andrew Hill2, Maria Blanca Hadacek3, Yvonne van Delft4 and Christiane Moecklinghoff5

1Infectious Diseases Unit, Medical Centre, Goethe-University Hospital, Theodor-Stern-Kai 7, Frankfurt, Germany; 2Janssen R&D, High Wycombe, UK; 3Janssen, Issy-les-Moulineaux, France; 4Janssen EMEA, Tilburg, The Netherlands; 5Janssen EMEA, Neuss, Germany

*Corresponding author. Tel: +49-69-6301-7680; Fax: +49-69-6301-5712; E-mail: c.stephan@em.uni-frankfurt.de

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Sir,

The Roche Amplicor HIV-1 Monitor™ Test, v1.5, UltraSensitive assay has been used to measure HIV-1 RNA levels in HIV clinical trials, but this assay has recently been discontinued. The new replacement assay, Roche TaqMan HIV-1 Test, v2.0, tends to show low levels of quantifiable HIV-1 RNA in samples undetectable by the Roche Amplicor UltraSensitive assay. An alternative assay is the Abbott RealTime HIV-1 assay, which has a lower quantification limit of 40 copies/mL.

Both the Roche Amplicor UltraSensitive assay and the Abbott RealTime assay can detect traces of HIV-1 RNA, below the standard quantification limits. Recent reports have suggested that low levels of detectable HIV-1 RNA may be a predictor of virological failure, particularly for patients receiving protease inhibitor monotherapy.

The aim of this study was to compare the performance of the Roche Amplicor UltraSensitive assay (used in the MONET trial) and the Abbott RealTime assay (used in the PROTEA trial) in plasma samples with HIV-1 RNA <200 copies/mL.

The MONET and PROTEA trials recruited patients with screening plasma HIV-1 RNA <50 copies/mL; patients were tested at screening then re-tested at the baseline visit, 4–6 weeks later. During both trials, patients remained on stable triple antiretroviral combination treatment between screening and baseline. The Roche Amplicor UltraSensitive assay was used for routine measurement of HIV-1 RNA in the MONET trial. Using this assay, HIV-1 RNA results were classified as: (i) no HIV-1 RNA detected; (ii) HIV-1 RNA <50 copies/mL, but virus detected under the quantification limit; or (iii) HIV-1 RNA ≥50 copies/mL.

The Abbott RealTime assay was used for routine measurement of HIV-1 RNA in the ongoing PROTEA trial. Using the Abbott assay, HIV-1 RNA results were classified as: (i) no HIV-1 RNA detected; (ii) HIV-1 RNA <40 copies/mL, but virus detected under the quantification limit; or (iii) HIV-1 RNA ≥40 copies/mL.

During the randomized phase of both trials, samples with low-level elevations in HIV-1 RNA (50–100 copies/mL in MONET and 40–200 copies/mL in PROTEA) were re-tested using the same assay. In addition, 122 samples with HIV-1 RNA <200 copies/mL by the Roche Amplicor UltraSensitive assay were re-tested with the Abbott RealTime assay.

In the MONET trial, there were 253 patients with both screening and baseline HIV-1 RNA data available using the Roche Amplicor UltraSensitive assay. Of the 247 patients who had HIV-1 RNA <50 copies/mL at screening, 237 (96%) had HIV-1 RNA <50 copies/mL at the baseline visit. There were six patients with HIV-1 RNA initially ≥50 copies/mL at screening (initially screening failure), of whom 3 (50%) had HIV-1 RNA <50 copies/mL at baseline. Between the screening and baseline visits, there was substantial fluctuation between the categories of ‘HIV-1 RNA not detected’ and ‘HIV-1 RNA <50 copies/mL, detected’ (Table 1). Of the 212 patients with HIV-1 RNA not detected at screening, 27 (13%) had HIV-1 RNA detected under the quantification limit at baseline, while 7 (3%) had HIV-1 RNA ≥50 copies/mL at baseline. Of the 253 patients tested overall, 188 (74%) had HIV-1 RNA in the same category at both screening and baseline. Given the fluctuations in HIV-1 RNA between the categories of ‘HIV-1 RNA not detected’ and ‘HIV-1 RNA <50 copies/mL, detected’, individual patient results in these two categories need to be interpreted with caution.

During the MONET trial, 36 samples with HIV-1 RNA initially between 50 and 100 copies/mL were re-tested with the same Amplicor UltraSensitive assay; the repeat sample showed HIV-1 RNA <50 copies/mL in 25 patients (69%). Of the 11 quantifiable re-test results, all showed HIV-1 RNA levels <200 copies/mL.

In the PROTEA trial, there were 299 patients with consecutive HIV-1 RNA measurements taken at the screening and baseline visits. Of the 292 patients who had HIV-1 RNA <40 copies/mL at screening using Abbott RealTime, 289 (99%) had HIV-1 RNA <40 copies/mL at baseline. There were seven patients with HIV-1 RNA >40; all seven patients had HIV-1 RNA <40 copies/mL at the baseline visit. Between the screening and baseline visits, there was also substantial fluctuation between the HIV-1 RNA categories (Table 2). Of the 246 patients who had HIV-1 RNA not detected at screening, there were 30 patients (12%) with HIV-1 RNA detected, but <40 copies/mL at baseline, and 1 patient (0.4%) with HIV-1 RNA >40 copies/mL at baseline. Of the 299 patients tested overall, 226 (76%) had HIV-1 RNA in the...
same category at both screening and baseline. Given the relatively high rates of fluctuation between the categories of ‘HIV RNA not detected’ and ‘HIV RNA <40 copies/mL, detected’, individual patient results need to be interpreted with caution.

During the PROTEA trial, there have been 44 post-baseline samples with HIV-1 RNA initially 40–200 copies/mL re-tested; 24 (55%) had repeat samples with HIV-1 RNA <40 copies/mL. Of the 20 quantifiable results, all showed HIV-1 RNA re-test results <200 copies/mL (data not shown).

Finally, there were 122 samples tested in parallel using the Roche UltraSensitive and Abbott RealTime assays; 112 (92%) were below the quantification limits of both assays. There were five samples with HIV-1 RNA ≥40 copies/mL by both Roche RealTime, but <50 copies/mL by Roche UltraSensitive. Conversely there were two samples with HIV-1 RNA ≥50 copies/mL by Roche UltraSensitive, but <40 copies/mL by Abbott RealTime. Of the 122 samples tested, 89 (73%) were in the same HIV-1 RNA category by both tests (Table 3). The limitation of this analysis is that the 122 samples were pre-selected as having low HIV-1 RNA levels by the Roche assay. This analysis should be repeated on a new set of samples, tested in parallel using the two assays.

In conclusion, this analysis suggests that plasma HIV-1 RNA levels in patients remain consistently <200 copies/mL between two consecutive study visits while on constant triple combination treatment. This consistency of HIV-1 RNA levels was seen when samples were evaluated by either the Roche Amplicor UltraSensitive assay or the Abbott RealTime assay. However, 1%–4% of patients had HIV-1 RNA below the quantification limit of 40–50 copies/mL at screening and then above quantification limits at baseline. Fluctuations over time between the categories of 'HIV-1 RNA not detected' and detectable or quantifiable levels of HIV-1 RNA were seen in 12%–16% of patients in the two trials. Samples with HIV-1 RNA of 40–200 copies/mL by either assay frequently had levels under the quantification limit after re-testing the same sample. The results suggest that any sample with initially low-level HIV-1 RNA should be re-tested to confirm whether this elevation is reproducible. Re-tested low-level HIV-1 RNA results were consistently <200 copies/mL in this study.

The performance of the Abbott RealTime HIV-1 assay was very similar to that of the Roche Amplicor HIV-1 Monitor Assay, suggesting that the Abbott assay could be used as a replacement for the discontinued Roche assay, without systematically changing the reporting of HIV-1 RNA results. This is important for the interpretation of results from new studies compared with those originally conducted using the Roche UltraSensitive assay. However, this study did not evaluate the reproducibility of test results on duplicate samples using the same assay. Changes in HIV-1 RNA levels between screening and baseline could result from the error of the assays as well as natural fluctuations in HIV-1 RNA levels over time in patients.

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Impact of test methodology, media type and ion content on the susceptibility of Acinetobacter spp. to tigecycline

Junyan Qu, Zhiyong Zong, Rujia Yu, Tianyu Hu, Hui Ye and Xiaoju Lü*

Center of Infectious Disease, West China Hospital, Sichuan University, Chengdu, China

*Corresponding author. Center of Infectious Disease, West China Hospital, Sichuan University, 37 Guoxue Lane, Chengdu 610041, China. Tel/Fax: +86-28-8542-3212; E-mail: lvxj33966@126.com

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Sir, Acinetobacter spp. are important opportunistic pathogens that mainly cause healthcare-associated infections. Although in recent years many studies have found that tigecycline exhibits antimicrobial activity against Acinetobacter,1-2 tigecycline-resistant Acinetobacter isolates have also been identified.3 Discrepancies in MICs have been reported when different methods or commercial Mueller – Hinton (MH) media have been used for in vitro susceptibility tests.4-7 Many studies have shown different tigecycline susceptibilities for Acinetobacter spp. between broth microdilution and the Etest or disc diffusion.5,6 We compared the susceptibilities of Acinetobacter spp. to tigecycline determined using agar dilution versus the broth microdilution method and then attempted to investigate the impact of the ion content of different media on tigecycline MICs for clinical Acinetobacter spp.

A total of 82 non-duplicate isolates of Acinetobacter spp. were collected, consisting of 67 Acinetobacter baumannii, 13 Acinetobacter pittii and 2 Acinetobacter nosocomialis. They were taken from patients from 13 hospitals in Sichuan, West China. Species identifications were established by partial sequencing of the recA gene.8 The MH agar (MHA) was purchased from Hopebio (Qingdao, China) and Oxoid (Hampshire, UK). MH II broth (MHb II; Becton, Dickinson (BD), USA) was used for broth microdilution. The susceptibility of each isolate was determined using both the agar dilution and broth microdilution methods, following CLSI guidelines.9 The FDA-approved tigecycline breakpoints for the Enterobacteriaceae (susceptible ≤2 mg/L; intermediate = 4 mg/L; resistant ≥ 8 mg/L) were used as provisional MIC breakpoints for the Acinetobacter isolates in this study. Each of the susceptibility tests for each isolate was performed in triplicate.

The resistance rate to tigecycline was high (47.56%) by the agar dilution method using MHA from Oxoid. However, no tigecycline-resistant isolate was found using MHA from Hopebio. The resistance rate of Acinetobacter spp. to tigecycline was found to be 19.51% by the broth microdilution method, similar to that in previous studies.5,7 A previous study had found that 86.2% of Acinetobacter isolates were susceptible to tigecycline using MHA (BD) by disc diffusion, but that only 28.5% were susceptible when using MHA (Oxoid).3 Tigecycline susceptibility results for Acinetobacter spp. should therefore be interpreted with caution.

The correlation between the agar dilution and broth microdilution methods was tested using Spearman’s rank correlation (SPSS). The tigecycline MICs determined using the agar dilution method (Oxoid/ Hopebio) were significantly correlated with those obtained by broth microdilution (BD) (P < 0.001). However, the MICs determined using agar dilution (Oxoid) were higher (mean 1.56-fold dilution) than those found by broth microdilution, and the MICs determined using agar dilution (Hopebio) were consistently lower (mean 1.04-fold dilution) than those determined using broth microdilution. Thamlikkul and Tiengrim2 also found that the inhibition zone diameters obtained using MHA (Oxoid) were consistently smaller than those obtained with MHA (BD). Different MICs were observed using different media even when using the same testing method, so the discrepancy might be related to the medium used. Discrepancies in the content of different media may have affected bacterial growth or the role of antimicrobial agents.

The concentrations of Ca, Mg, Mn and Zn ions in the three media were determined using atomic absorption spectroscopy (Varian Spectr AA-200). Spectroscopic measurements were carried out at the Analytical and Testing Center in Sichuan University. The content of manganese in Hopebio MHA...